PATENT SPECIFICATION

748,857

Date of Application and filing Complete Specification: Dec. 16, 1953.
No. 35033/53.
Application made in Norway on Dec. 16, 1952.
(Patent of Addition to No. 699,677 dated March 25, 1952).
Complete Specification Published: May 9, 1956.

Index at acceptance:—Class 81(1), B2(C:D:J:L:Q:Z).

COMPLETE SPECIFICATION

Process for the making of Adrenocorticotrophin (ACTH) Preparations having a prolonged action from ACTH with High Specific Activity

We, NYEGAARD & Co. A/S, of 103, Sandakerveien, Oslo, Norway, a Body Corporate organized under the laws of Norway, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the making of adrenocorticotrophin (ACTH) preparations having a prolonged action, and is an improvement in or modification of the invention of Specification No. 699,677.

In the parent Specification a process is described for making ACTH preparations for implantation or injection intramuscularly or subcutaneously to give a prolonged action, wherein the adrenocorticotrophin is converted into a stable suspension by adding an alkaline protein or protein degradation product, preferably protamine sulphate, and a water-soluble zinc salt to an adrenocorticotrophin solution in water, and adjusting the pH of the solution to between 6 and 8 by the addition of a buffer salt or a buffer salt mixture.

It has now been found that when this method is followed using ACTH preparations with a high specific activity, e.g. such preparations as have been purified by means of oxycellulose (cf. Astwood, Raben, Payne & Grady, Journal of the American Chemical Society, *73*, 2969 (1951)), the product obtained will show a considerably shorter clinical action than expected, if the method is carried out in the manner indicated in the examples of the said Specification.

The method which is the subject of the present invention represents a modification of the process described in the parent Specification, according to which, by using ACTH preparations with high specific activity, the desired prolonged action is attained with certainty. To make it easier to understand the invention the adrenocorticotrophin and the refined adrenocorticotrophin having high specific activity will be described more fully.

Adrenocorticotrophin is, as is known, a hormone defined by its biological effects, and according to the current view exists in association with proteins or peptides having varying molecular weights, depending upon the method used in making. Whether the active principle is represented by a definite low-molecular weight molecule, which in raw extracts and refined preparations is found associated by adsorption forces with foreign proteins and/or peptides, or whether in the natural starting material it constitutes a chemical group in one protein or several proteins, which can be degraded by chemical isolation and purification while retaining their activity, is at present unknown. It has been found, however, that, while the so-called pure protein hormone isolated by Li *et al.* (J. Biol. Chem. 149, 413 (1943)) and by Sayers *et al.* (J. Biol. Chem. 149, 425 (1943)) according to two different methods, with a molecular weight of about 20,000, has a specific activity of about 1.0 I.U./mg. (I.U. = International Units) and isoelectric point at 4.7, ACTH preparations can be made by purification by means of ion-exchangers (Dixon *et al.*, Nature *168*, 1084 (Dec. 22, 1951), 1044 (Dec. 15, 1951)), counter current distribution of oxycellulose (Astwood *et al.*, loc. cit.), with high specific activity and with isoelectric point on the alkaline side of the neutral point.

By persons skilled in the art it has been urged that, although ACTH of relatively low specific activity (about 1 I.U./mg.) with isoelectric point at pH<7 can indeed give sparingly soluble complex compounds with zinc protamine, this is not the case with ACTH of higher specific activity, e.g. such as is obtained by purification with oxycellulose. This is, however, incorrect.

We have found by experiments that such purified ACTH preparations with high specific

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activity, when treated with protamine, zinc salt and trisodium phosphate in the manner described in the parent Specification do in fact give sparingly soluble complex compounds with zinc protamine, the clear solution obtained after centrifuging of the suspension so formed being only slightly active, while almost the full activity is found again in the suspended particles.

Clinical tests have shown, however, that the prolonged effect (duration of the clinical action) depends on the concentration at which the suspension is formed, so that with ACTH preparations having high specific activity, relatively little prolonged action is obtained when extremely diluted aqueous ACTH solutions are used, while by forming the suspension from suitably concentrated aqueous solutions larger particles are formed and thereby a prolonged effect is obtained which is not inferior to that of similar preparations made from ordinary ACTH with an activity of about 1 I.U./mg. Thus it has been found that the suspension which is formed according to the process of the parent Specification from ACTH with an activity of about 0.8 I.U./mg. by means of the reagents and at the concentrations stated in the example in the parent Specification contains mainly ACTH zinc protamine particles or particle aggregates having a diameter of about 3/100 mm. Besides, some smaller particles with a diameter of about 1/1000 mm are found under the microscope.

In the example given in the parent Specification an amount of ACTH corresponding to 550 mg of the standard preparation was employed, which after the adoption of international units corresponds to 550 I.U.

However, under exactly the same conditions, with an ACTH preparation having an activity of about 10 I.U./mg (using the same concentrations as regards activity), a suspension is obtained in which the ratio between small and large particles is completely altered, most of the particles having a diameter of about 1/1000 mm, and only a small proportion measuring about 3/100 mm. Such preparations give considerably shorter clinical action.

It has now, in accordance with the invention, been found that when an ACTH preparation of, for example, the last mentioned type (activity about 10 I.U./mg) is precipitated in a more concentrated aqueous solution as regards its activity, for example as stated in Example 1 below, most of the particles (about 75%) will have a diameter of about 3/100 mm to 5/100 mm, while only a relatively small number have a diameter of 1/1000 mm.

Accordingly the present invention provides a process for making adrenocorticotrophin preparations for implantation or injection intramuscularly or subcutaneously to give a prolonged action, wherein an aqueous solution of purified adrenocorticotrophin of specific activity greater than 2 I.U./mg., which solution is of relatively high concentration as regards its activity, is converted into a stable suspension by adding to the solution a protamine sulphate and a water-soluble zinc salt and adjusting the pH of the solution to between 6 and 8 by the addition of a buffer salt or a buffer salt mixture.

The concentration of the aqueous adrenocorticotrophin solution used, expressed in I.U. per ml., at the end of the formation of the stable suspension preferably exceeds an amount corresponding to five times the specific activity expressed in I.U./mg. of the adrenocorticotrophin. The concentration of the aqueous protamine sulphate solution should exceed 1% and preferably exceed 2%.

Microscopic examination indicates that the larger particles are built up of particles of the smaller type (about 1/1000 mm.). Preparations of this type show as long clinical action as preparations made in the manner previously described from ACTH with an activity of about 0.8 I.U./mg.

In one embodiment of the invention the procedure is to dissolve the adrenocorticotrophin in part of the aqueous protamine sulphate solution and from this to form a suspension by adding a corresponding quantity of aqueous zinc sulphate solution and buffer salt solution until the pH attains any desired value between 6 and 8, whereupon the remainder of the protamine sulphate solution is added, and the remainder of the zinc salt solution together with the buffer salt solution is slowly added in such a way that the pH, during the simultaneous addition of the last-mentioned reagents, diverges as little as possible from the desired final pH between 6 and 8.

Below are given some examples of ways of carrying out the process according to the invention:

EXAMPLE 1.

A solution of 0.6000 g. ACTH with specific activity 10 I.U/mg. (which amount thus corresponds to 6000 I.U. ACTH) in 40.00 ml. of water is added to 33.00 ml. of aqueous 6% protamine sulphate solution. Then 5.12 ml. of 20% aqueous zinc sulphate ($ZnSO_4.7H_2O$) is added, followed by 1.20 ml. of 20% aqueous glucose solution. The pH is adjusted to 6.6 by the addition of 8.00 ml. of 20% aqueous trisodium phosphate ($Na_3PO_4.12H_2O$), 0.90 g. of tricresol is added, and the suspension made up to 300.0 ml. with 212.0 ml. of distilled water. The making is effected with sterile solutions and with sterile precautions.

EXAMPLE 2.

0.107 G. of adrenocorticotrophin with specific activity 56 I.U./mg., which amount thus corresponds to 5.992 I.U. ACTH, is dissolved in 10 ml. of 6% aqueous protamine sulphate solution. Then 1.55 ml. of 20% aqueous zinc sulphate ($ZnSO_4.7H_2O$) is added,

followed by 1.20 ml. of 20% aqueous glucose. The pH is adjusted to 6.6 by adding 2.3 ml. of 20% aqueous trisodium phosphate ($Na_3PO_4.12H_2O$) while stirring. Then a further 23 ml. of 6% protamine sulphate solution is added, after which 3.57 ml. of 20% zinc sulphate solution and sufficient (5.6 ml.) 20% trisodium phosphate solution are added simultaneously drop by drop, while stirring, so that the pH the whole time is near the final value of 6.6. The suspension is diluted with 250 ml. of distilled water; the pH is again adjusted to 6.6 with a few drops of N/10 hydrochloric acid and trisodium phosphate, and after the addition of 0.90 g. of tricresol the suspension is made up to 300 ml. with 2 ml. of distilled water.

The process according to the invention is, of course, not confined to the embodiments exemplified here, it being possible to alter the ratio of concentrations stated, depending on the ACTH preparation used, the essential feature of the invention being that the suspension is formed from such concentrated aqueous solutions that most of the particles obtained are relatively large.

What we claim is:—

1. A process for making adrenocorticotrophin preparations for implantation or injection intramuscularly or subcutaneously to give a prolonged action, wherein an aqueous solution of purified adrenocorticotrophin of specific activity greater than 2 I.U/mg., which solution is of relatively high concentration as regards its activity, is converted into a stable suspension by adding to the solution a protamine sulphate and a water-soluble zinc salt and adjusting the pH of the solution to between 6 and 8 by the addition of a buffer salt or a buffer salt mixture.

2. Process as claimed in claim 1, wherein the concentration of the aqueous adrenocorticotrophin solution employed, expressed in I.U. per ml., at the end of the formation of the stable suspension exceeds an amount corresponding to five times the specific activity in I.U./mg. of the adrenocorticotrophin.

3. Process as claimed in claim 1 or 2, wherein an aqueous solution of protamine sulphate is used and the concentration thereof exceeds 1% and preferably exceeds 2% by weight.

4. Process as claimed in any of the preceding claims wherein the adrenocorticotrophin is dissolved in the aqueous protamine sulphate solution.

5. Process as claimed in any of the preceding claims wherein the adrenocorticotrophin is dissolved in a part of the aqueous protamine sulphate solution and a suspension is formed by adding a corresponding quantity of aqueous zinc sulphate solution and buffer salt solution until the pH attains any desired value between 6 and 8, whereupon the remainder of the protamine sulphate solution is added, and the remainder of the zinc salt solution together with the buffer solution is slowly added in such a way that the pH during the simultaneous addition of the last mentioned reagents diverges as little as possible from the desired final pH between 6 and 8.

6. A process of making adrenocorticotrophin preparations substantially as described with reference to either of the examples.

7. Adrenocorticotrophin preparations for implantation or injection intramuscularly or subcutaneously to give a prolonged action, whenever produced by the process claimed in any of the preceding claims.

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Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.—1956.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.